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Review

Transgenic animals relevant to Alzheimer's disease

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Abstract

This article reviews the functional studies that have been carried out on transgenic and knockout animals that are relevant to Alzheimer's disease (AD). The discussion focuses upon the functional characterisation of these strains, particularly upon factors that affect synaptic processes that are thought to contribute to memory formation, including hippocampal long-term potentiation. We examine the use of transgenes associated with amyloid precursor protein and presenilin-1, their mutations linked to early onset familial AD, and the recent attempts to establish double transgenic strains that have an AD-like pathology which occurs with a more rapid onset. The development of new transgenic strains relevant to Alzheimer's disease has rapidly outpaced their characterisation for functional deficits in synaptic plasticity. To date most studies have focused on those transgenes linked to the minority of familial early onset rather than late-onset sporadic AD cases, and have focused on those changes linked to the induction of the early-phase of hippocampal long-term potentiation. Future studies will need to address the question of whether the development of AD pathology can be reversed or at least halted and this will be aided by the use of conditional transgenics in which genes linked to AD can either be switched on or off later in development. Furthermore, it remains to be resolved whether the deficits in synaptic function are specific to the hippocampus and whether deficits affect late-phase long-term potentiation. Nonetheless, the recent advances in genome sciences and the development of transgenic technology have provided a unique opportunity to study how genes associated with human cognitive dysfunction alter synaptic transmission between neurones in the mammalian brain. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Transgenic; Knockout; Alzheimer's disease; Hippocampal; Down syndrome

1. Historical overview

Alzheimer's disease (AD) was named after the physician Alois Alzheimer who in 1907 reported the case of an elderly female patient who had severe cognitive impairments and a characteristic pathology within the brain. Currently, AD and associated dementias affect approximately 10% of over 65 year olds and 30% of over 80 year olds and are the fourth leading cause of death amongst the elderly (e.g. Ebly et al., 1994; Lautenschlager et al., 1996). Consequently, there is an important scientific and clinical need to characterise the mechanisms that underlie the neuronal dysfunction associated with this disease so that new treatment strategies can be developed.

The initial symptoms of AD include poor memory retention of recent events and confusion about time and place. Subsequently, the disease progresses such that the patient exhibits impairments in speech, thought and memory, and is ultimately fatal (reviewed in Khatchaturian, 1985). Most studies of this disease have employed cross patient comparisons of the distribution and magnitude of various morphological (e.g. spine structure), and pharmacological (e.g. receptor subunit compositions) markers in post-mortem diseased brain with those in age-matched controls. However, the recent advances in genome sciences and the development of transgenic technology have opened up the possibility of studying alterations in synaptic transmission between different neuronal populations in strains of mice that carry genes associated with human cognitive dysfunction. These are likely to be complex in view of the multiple structural and biochemical changes that have

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been reported to accompany AD. In particular, the cellular processes underlying long-term plasticity of glutamate-mediated synaptic transmission, which is believed to be intimately involved in learning and memory processes in vivo, are likely to be profoundly affected. The rationale in developing transgenic animals relevant to AD is that they will be valuable in helping to construct a synaptic model for the memory impairments that are characteristic of AD, from which new treatment strategies can be formulated.

Morphologically AD is characterised by the deposition of amyloid plaques and neurofibrillary tangles in the cortex and hippocampus followed by neuronal and synaptic loss (reviewed in Khatchaturian, 1985). The neuritic plaques are extracellular lesions that are composed of the 40 to 42/3 amino acid long peptide $A\beta$ fragments derived from amyloid precursor protein (APP), whereas the neurofibrillary tangles are intracellular lesions composed of twisted filaments of tau protein. Approximately 10% of AD cases are classified as early onset in that they occur at ages of < 50 years. These cases are caused by autosomal dominant mutations in one of at least three genes. Two to three percent of early onset cases are linked to single point mutations in the gene which encodes amyloid precursor protein (Goate et al., 1991). These include the Swedish (APP670/671) and London (APP717) familial mutations (reviewed in Hardy, 1997). A larger proportion of early onset AD cases (70–80%) are linked to loci on chromosome 14, which correspond to presenilin-1 (PS-1; Sherrington et al., 1995). A structurally related protein, presenilin-2 (PS-2), encoded on chromosome 1, has also recently been identified and linked to AD (Rogaev et al., 1995). The precise function of these proteins is not known, however, it has been hypothesised that presenilins regulate APP processing and that the missense mutations in PS-1 (> 25 to date) affect the formation of $A\beta$. Consistent with this, it has recently been shown that the normal cleavage of amyloid precursor protein is dependent upon the expression of PS-1 (De Strooper et al., 1998).

A risk factor associated with AD has been linked to chromosome 19 and identified as apolipoprotein E (Pericak-Vance et al., 1991; Ropers and Pericak-Vance, 1991; Weisgraber et al., 1994; Meyer et al., 1998). ApoE is a 299 amino acid plasma protein which is found within the cytoplasm of many neurones within the central nervous system. ApoE has been implicated as a regulator of neuronal neurite outgrowth and of response to damage. There are two polymorphisms in the gene of ApoE which result in three isoforms termed ApoE2, ApoE3 and ApoE4. Those individuals with the ApoE4 isoform (ApoE C112- > R) have a higher risk of developing late-onset AD. Interestingly, ApoE2 has the opposite effect: it decreases the risk of developing AD and delays the onset of the disease. Like presenilin

proteins, there is evidence that ApoE is associated with APP in neurones. It binds to soluble $A\beta$ in vitro, is associated with amyloid plaques, and might promote $A\beta$ induced fibrillogenesis and microglial activation (Wisniewski et al., 1993; Barger and Harmon, 1997). An additional risk factor associated with AD has recently been identified as a polymorphic variant in the α-microglobulin A2M gene, which is a multifunctional lipoprotein receptor to which APOE and APP can bind (Blacker et al., 1998).

A large proportion of AD cases are sporadic and do not have a defined etiology. Nonetheless, a number of hypothesis have been proposed that may contribute to the disease. The generation of tau filaments may contribute to neuronal degeneration, since tau is involved in microtubule assembly and its abnormal metabolism is likely to lead to the disruption of cytoskeleton (Goedert et al., 1996). Furthermore, it is clear that in AD there is a substantial loss of cholinergic neurones within the forebrain. Agents which either slow down the degradation of acetylcholine by blocking the action of acetylcholinesterase, e.g. Tacrine and Aricept, or those which act as agonists on central muscarinic receptors can improve cognitive performance in animal models, and to a limited extent can improve cognitive performance in AD patients (e.g. Rogers et al., 1998). However, since these treatment strategies have limited efficacy, and have side effects, it is essential the degenerative processes that occur during AD are better understood so that more effective treatment strategies can be developed.

Whilst a link between abnormal APP metabolism and AD pathology has been established the question of what is the normal biological function of APP, or presenilin proteins, remains largely unanswered. Amyloid precursor protein (APP) is a single transmembrane spanning protein that is widely distributed in the CNS and peripheral tissues. Several studies have implicated APP or its fragments in the regulation of G-protein coupling, Ca^{2+} homeostasis, apoptosis, and as a regulator of cell growth and adhesion (Saitoh et al., 1989; Schubert et al., 1989; Milward et al., 1992; Qui et al., 1995; Perez et al., 1997). Similarly, although PS-1 has been implicated in trafficking and processing of proteins, including APP, within the endoplasmic reticulum and Golgi of neuronal cells, its normal biological role has yet to be clearly defined and this is essential if we are to understand its role in AD pathology.

Bi-directional changes in glutamate synaptic strength are believed to be important in the formation and consolidation of memory in vivo (Bliss and Collingridge, 1993; Malenka and Nicoll, 1993). Each form of synaptic plasticity is likely to be dramatically affected in animals exhibiting some of the features linked with AD since this condition is associated with alterations in, for example, glutamate receptor subunit

composition (Yasuda et al., 1995), efficiency of G-protein-linked receptor coupling to effectors (Roth et al., 1995), spine morphology (Wakabayashi et al., 1994) and inositol trisphosphate/nitric oxide production (Wallace 1994; Bonkale et al., 1995; Shimohama and Matsushima, 1995), all of which are important for different stages of the induction and maintenance of long-term potentiation (LTP) and long-term depression (LTD). The induction of LTP or LTD is critically dependent upon the pattern of the conditioning afferent input used to induce these phenomena, in particular, the integration of inhibitory and excitatory synaptic inputs. Likewise information processing within the hippocampus will be disrupted by abnormalities in the function of G-protein coupled receptors (e.g. $GABA_B$ and metabotropic glutamate receptors) implicated in the synchronisation of neuronal networks involved in the consolidation of memory processes (Whittington et al., 1995) as well as in the induction of LTP and LTD per se (Davies et al., 1991; Bashir et al., 1993; Bolshakov and Siegelbaum, 1994).

Research into the genetic basis, mechanism of neuronal degeneration, and future treatments and models of AD have been extensively reviewed elsewhere (Khatchaturian, 1985; Friedland, 1993; Weisgraber et al., 1994; Yankner, 1996; Hardy, 1997; Selkoe, 1997; Neve and Robakis, 1998; Sabbagh et al., 1998; Sirinathsinghji, 1998). In this article we focus upon the functional consequences of transgene expression particularly upon factors that affect synaptic processes thought to contribute to memory formation, the techniques and rationale behind the generation of transgenic animals with amyloid precursor protein and presenilin-1, and recent attempts to establish double transgenic strains with AD-like pathology that occurs with a more rapid onset.

2. Development of transgenic and knockout techniques

Transgenic mice, in the form of gene knockouts by the homologous recombination technique or random insertion of wildtype or mutant transgenes, are important tools that provide insight into the function of a gene in vivo and can provide models of disease states to test hypotheses for potential therapeutic intervention. Therefore, a considerable amount of effort has been undertaken by many laboratories to develop transgenic lines resembling key features of AD.

2.1. Generation of transgenic animals

Conventional gene targeting by the homologous recombination technique in embryonic stem cells has also been successfully applied to investigate the physi-

ological role of genes involved in the predisposition to AD. A complete inactivation of the mouse APP gene was achieved by deleting a DNA sequence encoding the APP promoter and its first exon including the AUG translation initiation codon and the signal peptide of APP (Zheng et al., 1995). Similar approaches to either interrupt or delete an exon(s) were chosen to generate gene knockouts of the presenilin 1 gene resulting in complete ablation of the PS-1 protein (Wong et al., 1997; Shen et al., 1997; De Strooper et al., 1998).

Transgenic mice harboring mutant forms of the APP and/or PS-1 gene associated with AD in humans are a valid tool to study the pathophysiological role of those genes in AD. Due to the relatively short life span of mice, of just 1-2 years, a high overexpression of the transgene is considered to be necessary to achieve the development of AD-like symptoms in these animals. Therefore, a strong, brain specific promoter is typically chosen to drive the transgene(s) of interest. Currently, those transgenic lines which are most successful were established using the platelet-derived growth factor (PDGF)-β promoter (Games et al., 1995), the promoter and regulatory regions of the PrP gene (Hsiao et al., 1996), and the murine Thy-1 promoter and exons (Sturchler-Pierrat et al., 1997). In all three lines a 7-10-fold overexpression of the transgenes was needed to develop features of AD-like pathology. The human transgenes used consisted either of a mutant minigene containing all exons plus some essential introns covering all three alternative splice forms of APP, or mutant cDNAs of the APP 695 and 751 form, respectively. There are also differences in the choice of the mutation associated with familial AD (APP(V717F) or APP(K670N + M671L) and the genetic background of the mice (Swiss Webster X B6D2F1, SJL X C57Bl/6 or C57Bl6). All these differences may account for the variations found in the pathology of the transgenic lines (see Section 3.2 and Table 1).

Transgenic lines overexpressing human PS-1 transgenes carrying FAD mutations were established by using the PDGF promoter (Duff et al., 1996), the mouse PrP promoter (Borchelt et al., 1996) the hamster PrP promoter (Citron et al., 1997) and the human Thy-1 promoter (Qian et al., 1998). In contrast to the highly overexpressing mutant APP transgenic lines (see above) the expression level of the mutant human PS-1 transgene is just 1-3-fold for all of the described PS-1 lines. Various mutations linked to early-onset cases of FAD have been engineered into the human PS-1 transgene, including the M146V, M146L and the A246E mutations, and have been introduced into different mouse strains to establish individual transgenic lines (see Section 4.2 and Table 2).

Table 1
APP-related knockout and transgenic mice

Gene	Amyloid/neuronal loss/behaviour	Effects on synaptic plasticity	Reference
Mouse APP knockout	Reactive gliosis, motor impairments Spatial learning deficits	Deficits in NMDA-dependent LTP in CA1	Zheng et al., 1995 Dawson et al., 1998 Seabrook et al., 1999
Mouse APP knockout Mouse APLP1 knockout	Normal	Not studied	Phinney et al., 1998 Muller et al., 1997
Mouse APLP2 knockout	Normal	Not studied	von Koch et al., 1997
Mouse APLP2 and APP knockout	Majority homozygous die at birth	Not studied	von Koch et al., 1997
Murine Aβ on mouse APP background	Aβ deposition, apoptotic neuronal loss, gliosis, increased mortality	Not studied	LaFerla et al., 1995
Human APP mutant (V717F)	Age-dependent Aβ elevation and plaque deposition	Not studied	Games et al., 1995 Johnson-Wood et al., 1997
	dystrophic neurites and apoptotic-like cells but no neuronal loss		Masliah et al., 1996 Irizarry et al., 1997
Human APP mutant	Gliosis, weak Aβ immunoreactivity, apoptotic-like cells, increased mortality	Not studied	Moechars et al., 1996
Human APP695 mutant (K670N+ M671L)	Age-dependent Aβ elevation, memory deficits and amyloid plaques		Hsiao et al., 1995, 1996
		Deficits in LTP in CA1 and DG	Chapman et al., 1997
Human APP751 wild-type	Age-dependent spatial learning deficits		Moran et al., 1995
Human APP751(K670N +M671L+V717I)	Amyloid plaques, hyperphosphorylated tau, and neuronal loss		Sturchler-Pierrat et al., 1997
Human APP(C100)	Amyloid deposition		Kammesheidt et al., 1992
	Hippocampal degeneration	Not studied	Oster-Granite et al., 1996
Human APP(C104)	Neuronal loss, deficits in spatial learning	Deficits in NMDA-dependent LTP in CA1	Nalbantoglu et al., 1997

2.2. New developments

An alternative method to cDNA constructs involves the use of yeast artificial chromosomes (YAC), which contain the whole APP or PS-1 gene including the FAD mutation and all regulatory elements in its chromosomal environment (Loring et al., 1996; Lamb et al., 1997). This approach of generating transgenic mice has the advantage of mimicking better the endogenous situation by achieving correct tissue specific expression of the gene of interest and by being more independent of the chromosomal integration site. However, DNA constructs of 400–1000 kb are fragile and consequently are more difficult to handle before pronuclear injection than smaller cDNA constructs. The YAC approach has also been successfully applied to generate transgenic rats carrying the Swedish mutation of the APP transgene (Folkesson et al., 1997). Transgenic rats may provide a more versatile model system to perform combined electrophysiological and behavioural studies. Therefore, the outcome of those analyses will be interesting to compare with results from existing transgenic mouse lines.

Another way to ensure tissue specific and endogenous expression level applies the homologous recombination technique in combination with the cre/loxP recombination system to introduce a FAD mutation directly into the endogenous APP mouse gene (Reaume et al., 1996). In this pointlox, or knockin, procedure a replacement vector containing the mutation and the neomycin resistance gene cassette flanked by loxP sites (floxed) is utilised. The floxed neo gene, whose expres-

Table 2
PS1 knockout and transgenic mice

Gene	Amyloid/neuronal loss/behaviour	Effects on synaptic plasticity	Reference
Mouse PS-1 knockout	Homozygous lethal in embryogenesis, PS-1 required for Notch1 and Dll1 expression	Not studied[a]	Wong et al., 1997
Mouse PS-1 knockout	Homozygous lethal in embryogenesis, abnormal neurogenesis, skeletal formation	Not studied[a]	Shen et al., 1997
Mouse PS-1 knockout	Homozygous lethal in embryogenesis, embryonic brain cultures leads to loss of cleavage of APP at γ secretase site	Not studied[a]	De Strooper et al., 1998
Human PS-1 mutant	Increase $A\beta$1-42/43 production	Not studied	Duff et al., 1996
Human PS-1 mutant	Increase $A\beta$1-42/43 production	Not studied	Borchelt et al., 1996
Human PS-1 mutant	Increase $A\beta$1-42/43 production	Not studied	Citron et al., 1997
Human PS-1 mutant (A246E)	Increase $A\beta$1-42/43 production	Increased in CA1 region	Borchelt et al., 1997a; Qian et al., 1998
Human PS-1 mutant on PS-1-null background	Rescues lethality seen in PS-1 null mice	Not studied	Davis et al., 1998; Qian et al., 1998

[a] Not studied in heterozygotes which are viable.

sion might interfere with the expression of the mutated gene of interest, can be removed by transient transfection of the cre recombinase in the targeted ES cells. Cre excised targeted ES cells are then introduced into blastocysts to generate chimeric animals, which are then bred for germ line transmission of the targeted mutation. Alternatively, the floxed neo gene cassette can be deleted in vivo by crossing a gene targeted mouse derived from a non-Cre excised ES clone with a transgenic mouse carrying the Cre recombinase (deleter mouse; Schwenk et al., 1995).

2.3. Importance of genetic background

Another important issue in the generation of transgenic lines is the genetic background. For example, the same human APP695(K670N + M671L) transgene caused early death in the inbred strain FVB/N, but led to the establishment of the successful transgenic line in the hybrid strain C57Bl/6-SJL (Hsiao et al., 1995, 1996). Moreover, outbreeding of the C57Bl/6-SJL background to the inbred C57Bl/6 strain resulted in much higher mortality rates of transgenic animals in the N3 (~ 94% C57Bl/6- 6% SJL) and, even more, in the N4 (~ 97% C57Bl/6- 3% SJL) generation (Carlson et al., 1997). Therefore, modifier genes of a certain genetic background can have a great influence on the phenotype of the transgenic mouse line, and, in the case of the APP695(K670N + M671L) mouse, a 50% C57Bl/6- 50% SJL ratio seems to be a more favourable genetic background. Hence, introduction of the transgene into various genetic backgrounds by pronuclear injection into different strains or outbreeding to different inbred strains may be the way to find out empirically the most suitable genetic background of a transgenic line preserving the phenotype and having the least undesired side effects like

high mortality rate, aggressiveness and breeding problems.

The genetic background can also have a great influence on the phenotype of gene targeted mice (Gerlai, 1996; Silva et al., 1997). As gene targeted mice are mainly being generated using embryonic stem cells of the polymorphic 129 substrain, random segregation of these polymorphic loci to either mutants or controls could affect the phenotype of the resulting animals and hamper the interpretation of the experiments. Hence, mutations should be ideally maintained as standard congenic lines by backcrossing over many generations onto defined inbred backgrounds. However, this process is expensive and time consuming, but can be speeded up to a degree by marker-assisted breeding schemes (Markel et al., 1997) and superovulation of prepubertal females (Behringer, 1998). Nevertheless, it is a difficult task to outbreed the regions flanking the mutation, as they are closely coupled to the targeted mutation.

2.4. Nomenclature

A consistent naming system for the transgenic lines generated by homologous recombination or random insertion has been suggested by the international committee on standardised genetic nomenclature for mice (Mouse Genome 92, 1994). In this review we have used a simplified nomenclature based upon the species gene product and mutation, e.g. the PDAPP mouse is referred to as the human APP(V717F) mutant transgenic mouse (Games et al., 1995), and the Tg2576 as the APP695(K670N + M671L) mutant transgenic mouse (Hsiao et al., 1996). Since differences in genetic background, the transgene promoter, or site of transgene incorporation can affect the phenotype of transgenic animals, when discussing strains that have been generated by different laboratories with similar genotypes we

have cited the transgenic strain and the initial publication in parenthesis.

3. Amyloid precursor protein

The β-amyloid peptide $(A\beta)$ is a 39–43 amino acid peptide and is a major component of senile plaques (Glenner and Wong, 1984). Its precursor, amyloid precursor protein, is a 695–770 amino acid long protein with a single transmembrane spanning region and is found in many cell types throughout the body (Kang et al., 1987).

There are at least six mRNAs produced by splice variants of the APP gene, which is located on chromosome 21 in humans and on chromosome 16 in mice. Of these splice variants APP695, APP751, and APP770 are the most prevalent isoforms. APP695 differs from that of APP751 and APP770 in that the latter have a domain homologous to the Kunitz-type serine protease, and APP695 is predominantly expressed in neurones. APP is expressed in the cell membranes of most cells where it has been hypothesised to be involved in several biological processes including the regulation of G-protein coupling, Ca^{2+} homeostasis, apoptosis, and as a regulator of cell growth and adhesion (Saitoh et al., 1989; Schubert et al., 1989; Milward et al., 1992; Qui et al., 1995; Perez et al., 1997). APP is metabolised by at least 3 enzymes at sites which are referred to as the α, β, and γ secretase sites to form a number of peptide fragments. These fragments include the $A\beta$ peptide which is a primary constituent of AD plaques (Fig. 1). As yet, none of the published data prove the existence of single and specific enzymes for the α, β, or γ secretase sites.

Enzyme activity at the α-secretase site cleaves APP within the $A\beta$ domain resulting in the generation of secretary APP (sAPPα) and the p3 C-terminal fragment (p3CT), a process which likely occurs in a post-Golgi-compartment. Cleavage at the β-secretase site in the more N-terminal part of the $A\beta$ domain, generate sAPPβ and A4CT fragments. Both A4CT and p3CT are cut by the γ-secretase within their transmembrane domain resulting in the release of $A\beta$ $(A\beta 1$-40 and $A\beta 1$-42 or the 16 residues shorter p3 peptide (p3-40 and p3-42) into the extracellular medium. Under normal conditions α-secretase cleavage is favoured, while in familial early onset AD associated with the Swedish mutation, APP is preferentially metabolised by β-secretase, leading to the generation of $A\beta$ fragments (e.g. Haass et al., 1995).

Interestingly, mutations in the transmembrane domain of APP can alter the specificity of γ-secretase yielding to a higher ratio of the toxic $A\beta 1$-42 to the $A\beta 1$-40 (Suzuki et al., 1994; Lichtenthaler et al., 1997). However, it is not clear whether it is the generation and accumulation of the C terminal APP fragments and/or the generation of $A\beta 1$-42 that results in an AD-like phenotype (Fig. 2).

Five principal types of transgenic animals involving APP, and also PS-1, have been generated. These include (i) conventional gene knockout, (ii) knock-in of single point mutation(s) by homologous recombination into the endogenous gene, (iii) random insertion of intact or truncated transgenes carrying single point mutations that have been linked to early onset familial AD, (iv) rescue of the knockout mice by crossing with a transgenic line containing the wildtype or mutated APP or PS-1 transgene, and (v) double transgenics in which human APP mutant mice have been crossed with other transgenic lines associated with AD such as the human PS-1 mutants. The double transgenics involving human APP and PS-1 are reviewed later in this article.

3.1. APP knockouts

To investigate the function of APP and its metabolites a mouse strain has been generated that lacks any APP, by targeted disruption of the gene encoding APP (Zheng et al., 1995). These mice have marked reactive gliosis within brain tissue, and exhibit behavioural deficits that include decreased locomotor activity and forelimb grip strength suggesting that APP deficient mice may have impaired neuronal function. Indeed, in cognitive tests APP-null mice have deficits in spatial learning and hippocampal synaptic plasticity (Dawson et al., 1998). These deficits in synaptic plasticity are associated with both pre- and post-synaptic changes within the hippocampus (Seabrook et al., 1999). Pre-synaptically, there is a disruption of synaptophysin staining in a subset of mice and disruption of paired pulse depression of GABA mediated synaptic transmission. Post-synaptically, hippocampal pyramidal neurones have a smaller overall dendritic length and project less into the stratum radiatum, as seen with MAP2 staining and biocytin labeling of individual pyramidal neurones (Seabrook et al., 1999; Fig. 3). Similar effects have been found in transgenic mice overexpressing human FAD forms of APP, e.g. the human APP(V717F) mutant transgene

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Fig. 1. (Opposite) Schematic diagram of amyloid precursor protein and sites at which it is preferentially cleaved by proteases, including that of the $A\beta 1$-40 and $A\beta 1$-42/43 fragments (in red).

Fig. 2. (Opposite) Proposed metabolism of APP under normal conditions compared to that in early-onset Alzheimer's disease. In Alzheimer's disease it has been hypothesised that APP is preferentially metabolised via β secretase activity leading to the generation of soluble C99 peptide fragments. However, it remains to be determined whether it is the generation of the C99 fragment or the subsequent production of $A\beta 1$-42 by γ-secretase activity that is the primary factor leading to the common pathology seen in early-onset and sporadic late-onset AD. A disruption of the normal function of APP may also contribute to the cognitive deficits.
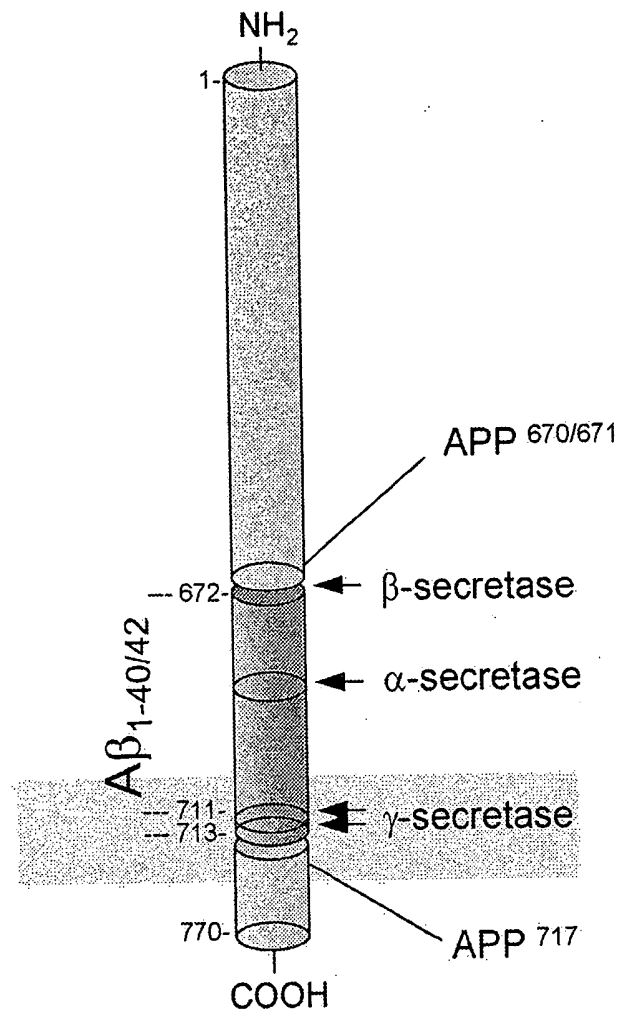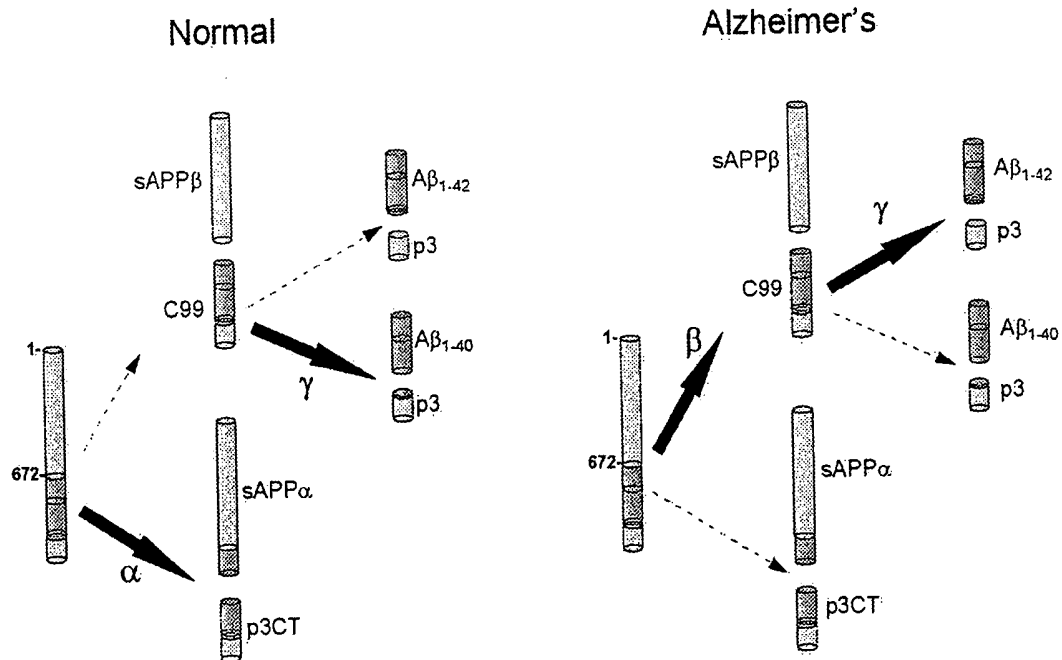
Fig. 1

Fig. 2

A) APP wildtype   B) APP-null

C

10 @ 100 Hz   4 x 10 @ 100 Hz

-o- APP WT (n = 20)
-•- APP -/- (n = 18)

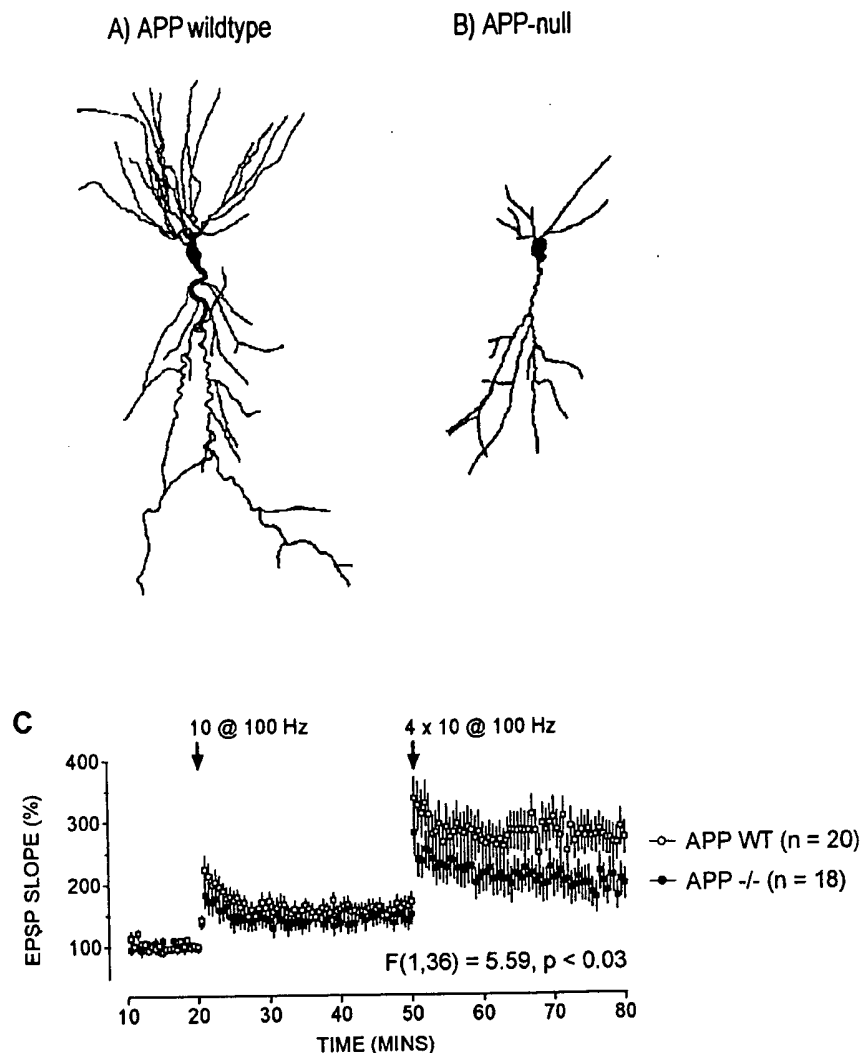F(1,36) = 5.59, p < 0.03

EPSP SLOPE (%)

TIME (MINS)

Fig. 3. Morphological characteristics of hippocampal neurones in the CA1 region of APP-null mice, and the deficits in induction of long-term potentiation (from Seabrook et al., 1999).

(Games et al., 1995). These results indicate that APP may be important for normal neuronal development, survival and/or function within the hippocampus. A disruption of normal APP function, due to abnormal proteolytic cleavage, could contribute to the functional deficits seen in AD. However, it is important to note that in APP-null mice deficits are observed only in a subpopulation of animals. Consequently, the absence of APP may simply be altering the susceptibility to phenomena such as seizures or neurotoxic damage. Indeed, if lethargic mice are excluded from functional characterisation there is no evidence for neuronal loss in 18–28 month old APP-null mice (Phinney et al., 1998). The variability found in the APP-null phenotype could be explained by assuming a functional compensation by the highly homologous APLP1 and APLP2 proteins. This view is supported by the finding that APLP2 null mice appear to have a mild phenotype, while most of the APP and APLP2 double knockout mice die at birth indicating a widely functional

redundancy of those two proteins (von Koch et al., 1997). Hence, it is unlikely that a disruption of the normal function of APP is the only mechanism that underlies the pathology in AD. Consequently, the results from studies using overexpression of transgenic APP containing mutations linked to familial early onset AD, particularly on an APP-null background, will be valuable to our understanding of the disease process.

3.2. APP mutants

These include transgenic mice expressing wild-type human APP751 on the mouse APP background (Moran et al., 1995), the truncated C-terminus of APP (Kammesheidt et al., 1992; Nalbantoglu et al., 1997), and mutations associated with early onset familial AD (FAD) including APP(V717F) (Games et al., 1995; Johnson-Wood et al., 1997), APP695(K670N + M671L) (Hsiao et

al., 1995, 1996), and APP751(K670N + M671L) (Sturchler-Pierrat et al., 1997; Calhoun et al., 1998). Several of these transgenic strains have a phenotype that include accelerated Aβ deposition, and to varying degrees, reactive astrocytosis, a disruption of neuronal morphology and/or number, and in some, but not all cases, behavioural and hippocampal deficits (Table 1). APP695(K670N + M671L) transgenic mice (Hsiao et al., 1996) have impaired spatial learning in the Morris watermaze, and exhibit deficits in LTP induction in the CA1 region, and dentate gyrus (Chapman et al., 1997). This phenotype is similar to that seen in APP-null mice, but not identical, since obviously there is no Aβ deposition in APP-null mice because the gene encoding the parent protein has been deleted. Another important difference between these APP695(K670N + M671L) transgenic mice, and APP-null mice, is that the former have a profound reduction in the efficiency of glutamate-mediate synaptic transmission whereas the deficits in APP-null mice are more subtle and may in part involve a disruption of GABA-mediated synaptic transmission (Seabrook et al., 1999). Nonetheless, it is possible that some of the functional deficits in FAD transgenic mice may be influenced by the disruption of normal APP function and are not just a consequence of Aβ deposition. Crossing the transgenic mice overexpressing a FAD mutation into the APP null background might provide support for this idea, if the offspring would show a more severe phenotype than the two parental strains alone.

Interestingly, transgenic animals expressing the truncated C-terminus of APP (Kammesheidt et al., 1992; Oster-Granite et al., 1996; Nalbantoglu et al., 1997), unlike transgenics expressing mutations associated with familial early onset AD, exhibit specific spatial learning deficits and neuronal loss. This has led to the argument that impaired cognitive function may be due to an accumulation of Aβ precursors rather than the generation of Aβ1-42 alone (e.g. Neve and Robakis, 1998). This may help explain the poor correlation between Aβ load and the severity of AD.

3.3. APP and Down syndrome

Subjects with Down syndrome have an additional APP allele and exhibit Aβ plaque deposition at early ages. The AD-like pathology seen in Down syndrome led to speculation that a gene on chromosome 21 was responsible for AD. Interestingly, the presence of soluble amyloid β-peptide, notably Aβ1-42, precedes amyloid plaque formation in Down syndrome (Teller et al., 1996) and it is this form of Aβ which is elevated in early onset familial AD. A mouse model of

Down syndrome, the Ts65Dn mouse strain, has recently been identified (Reeves et al., 1995). Ts65Dn mice have a triplicated segment of chromosome 16 that is syntenic to part of the Down syndrome region of human chromosome 21. These animals exhibit deficits in spatial working memory detected in mice 6 months old, however, these were much more pronounced at 12 months with performance only at chance levels even after 15 training sessions (one per day). At this age deficits in NMDA-dependent LTP formation is also evident within the CA1 region of the hippocampus (Siarey et al., 1997).

4. Presenilins

The majority of early-onset cases of familial AD are caused by mis-sense mutations in the presenilin genes, rather than in the APP gene itself. Two closely related genes, the presenilin-1 gene on chromosome 14 and the presenilin-2 gene on chromosome 1, have been identified (Sherrington et al., 1995; Slunt et al., 1995; Levy-Lahad et al., 1995; Rogaev et al., 1995). PS-1 and PS-2 are transmembrane proteins containing eight putative transmembrane domains. An endoproteolytic cleavage of presenilins occurs at a site near the loop domain, which seems to be important in activating presenilin function. The proteolytic processed presenilin-1 regulates the unusual intramembranous proteolysis of the amyloid precursor protein by γ-secretase (De Strooper et al., 1998). Therefore, the absence of presenilin-1 results in a decreased Aβ1-40, Aβ1-42 and p3 production, while missense mutations of presenilin-1 leads to an increased Aβ1-42 production (Haass and Selkoe, 1998). The presenilins are expressed throughout the brain and are localised primarily within the endoplasmic reticulum of cell bodies and dendrites.

4.1. PS-1 knockouts

Targeted disruption of the murine PS-1 gene is lethal during embryogenesis and is accompanied by hemorrhages in the CNS of the homozygous mutant mice (Wong et al., 1997; Shen et al., 1997; De Strooper et al., 1998). PS-1 null mice embryos also have abnormal formation of the axial skeleton and spinal ganglia. This results from a requirement of PS-1 in the normal spatiotemporal expression of *Notch1* and *Dll1*, which are essential for somite segmentation and maintenance of somite borders. PS-1 shares homology to the *C.elegans* gene sel-12, which is involved in *Notch* signaling, and which explains the similarity found between the phenotypes of PS-1 and

Notch knockout mice. To date few functional or electrophysiological recordings from neuronal cultures of homozygote or hippocampal slices of adult heterozygote PS-1 mice have been reported (Table 2).

4.2. PS-1 transgenics

Transgenic lines overexpressing mutant forms of a human PS-1 transgene on the mouse PS-1 background have been established by various groups (Duff et al., 1996; Borchelt et al., 1996; Citron et al., 1997; Qian et al., 1998). Although the choice of promoter, FAD mutation of the transgene and mouse strain differed, an increase of $A\beta$1-42/43 production was found in all lines indicating an important role of presenilin-1 in APP processing in vivo. The magnitude of increase in $A\beta$1-42 was greater in the M146L than in the L286V mutant PS-1 transgenic line (Citron et al., 1997). However, none of the mutant PS-1 transgenic lines investigated so far exhibit an abnormal pathology which could be explained by the smaller level of overexpression of the transgene than in the APP transgenic models (one to three times in comparison to eight to ten times, respectively) or by the different nature of mutations.

Transgenic mice over-expressing human PS-1(A246E) have enhanced LTP in the CA1 region of the hippocampus. LTP induced by either theta burst stimulation or by a 100 Hz tetanus is increased, but this is not accompanied by changes in fibre volley amplitude, field EPSPs or paired-pulse facilitation (Borchelt et al., 1997a). The mechanism underlying this change may involve a subtle decrease in buffering of post-synaptic calcium transients since the Ca^{2+}-dependent after-hyperpolarisation following action potential discharges is enhanced in CA1 neurones from PS-1(A246E) mice (Dr J.J. Jeffreys, personnal communication).

4.3. PS-1 rescue

The embryonic lethality of presenilin-1 deficient mice can be rescued by crossing the PS-1 null mice to human PS-1 transgenic lines (Davis et al., 1998; Qian et al., 1998). Interestingly, not only transgenic lines expressing the wildtype, but also the mutant presenilin-1 gene carrying the human PS1(A246E) mutation can rescue the severe phenotype of the PS-1 knockout mouse to similar degrees. This finding clearly indicates that the mutation of the presenilin 1 gene does not lead to a loss of function during mouse development. Remarkably, the level of $A\beta$1-42 and the ratio of $A\beta$1-42/40 are significantly increased in the human mutant PS-1 rescued PS-1 knockout mice than in the mutant PS-1 transgenics alone, although De Strooper et al. (1998) found a 5-fold decrease in the production of amyloid peptide in the PS-1 deficient embryos. This apparent paradox is best explained by assuming that the FAD

mutation of the presenilin-1 gene comprises a gain of function, which can be suppressed by competition of normal functioning murine PS-1 alleles, which are present in the human mutant PS-1 transgenic, but are absent in the PS-1 rescued mice.

5. Double transgenics

The current single transgenic animal models of Alzheimer's disease have either limited AD-like pathology, or in those that do have pathology it occurs at relatively late ages of around 9–12 months (see Sections 3.2 and 4.2). To facilitate the discovery of new drug treatments which may prevent $A\beta$ deposition several laboratories are actively developing transgenic mice that carry multiple genetic mutations involved in FAD. Whilst these double transgenics may not necessarily yield more information about the mechansims involved in AD pathology, they will be valuable since the onset of the pathology occurs at earlier ages. Indeed, the increase in the ratio of the highly amyloidogenic $A\beta$1-42 peptide relative to total $A\beta$ found in both transgenic lines overexpressing either mutant APP or mutant PS-1 is greatly enhanced in double transgenics carrying both mutant transgenes (Borchelt et al., 1996; Citron et al., 1997). Moreover, the AD-like phenotype is greatly accelerated in double transgenic mice (APP695(K670N + M671L) peptide × PS-1(A246E): Borchelt et al., 1997b; APP695(K670N + M671L) × PS-1(M146L): Holcomb et al., 1998). Large numbers of amyloid deposits in the cerebral cortex and hippocampus were found already starting at 6 months of age in the double transgenics in comparison to 9–12 months in the single APP695(K670N + M671L) mutant APP line alone, which is in good correlation with higher amounts and earlier onset of $A\beta$ levels in the double transgenics. Interestingly, both mutant APP and PS-1 double and APP single transgenic mice demonstrate a reduced spontaneous alternation performance in a 'Y' maze compared to mutant PS-1 single transgenic or wildtype control littermates at three months of age. Apparently, the behavioural deficits precede the amyloid plaque deposition. It needs to be shown, if the deficits in NMDA-dependent LTP in the CA1 region of the hippocampus, which were detected in APP single transgenic mice, occur earlier and/or are more profound in the APP and PS-1 double transgenic mice (Table 3).

6. Relevance to Alzheimer's disease

The recent advances in genome sciences and the development of transgenic technology have provided a unique opportunity to study how genes associated with human cognitive dysfunction alter synaptic transmis-

Table 3
APP and PS1 double transgenic mice

Gene	Amyloid/neuronal loss/behaviour	Effects on synaptic plasticity	Reference
Mo/Hu APP695(K670N + M671L) peptide × human PS-1; wt	Same $A\beta$ 1-42/43 production as APP695	Not studied	Borchelt et al., 1996, 1997b
Mo/Hu APP695(K670N + M671L) peptide × human PS-1; A246E	Enhanced $A\beta$ 1-42/43 production versus APP695	Not studied	Borchelt et al., 1996, 1997b
Human APP695 × human PS-1; wt	Same $A\beta$ 1-42/43 production as APP695	Not studied	Citron et al., 1997
Human APP695 × human PS-1; L286V	Slightly enhanced $A\beta$ 1-42/43 production versus APP695	Not studied	
Human APP695 × human PS-1; M146L	Enhanced $A\beta$ 1-42/43 production versus APP695	Not studied	
Human APP695(K670N + M671L) × human PS-1 (M146L)(K670N,M671L)	Enhanced $A\beta$ 1-42/43 production versus APP695	Not studied	Holcomb et al., 1998

sion between neurones in the mammalian brain. However, one of the difficult issues surrounding knockout and transgenic studies is the relevance of the animal phenotype to the disease state. In the case of models of Alzheimer's disease, mice have a short life span and therefore one can realistically question the relevance of changes seen over the space of a few years to a disease which, in humans, takes several decades to occur. It is similarly difficult to eliminate the influence that the disruption of the genotype of an animal may have upon developmental processes. However, the creation of inducible transgenics and knockout techniques will, to some extent, help to address these concerns. Clearly, both issues will remain a significant philosophical challenge for the interpretation of transgenic studies and must be considered carefully when extrapolating to human disease states. Nonetheless it is clear that transgenic studies have already provided, and will continue to provide valuable information regarding the significant role that APP and PS-1 mutations have in early-onset familial AD.

6.1. Relevance of changes in hippocampal synaptic plasticity to dementia

One of the principal symptoms of AD is a disruption of cognitive processes including a disruption of verbal, spatial, and visual learning and memory. Therefore, it is likely that changes in hippocampal synaptic function in transgenic animals relevant to AD will provide insights into the neurodegenerative changes that take place in the early stages of the disease and might provide a framework from which effective treatment strategies can be developed. The hippocampus is an important brain region involved in many, but not all, aspects of learning and memory. In humans, bilateral lesions of the hippocampal formation cause marked anterograde amnesia, which include verbal and non-verbal deficits (Scoville and Milner 1957; Zola-Morgan et al., 1986) similar to that seen in AD. Furthermore, neuropatholog-

ical studies have shown that in late stage AD there is pronounced neuronal loss within the layer II of the entorhinal cortex and hippocampal cell body layers, which is associated with the presence of amyloid plaques and neurofibrillary tangles (reviewed in Khatchaturian, 1985; Sirinathsinghji, 1998). Selective lesions within the CA1 cell body layer of the hippocampus are sufficient to induce learning deficits in both rodents and humans (e.g. Zola-Morgan et al., 1986), and consequently the majority of functional studies on transgenic animals have concentrated upon determining whether transgene expression affects synaptic plasticity in this brain region. Recent studies using functional magnetic resonance imaging have found that a large proportion of patients with mild cognitive impairment and hippocampal atrophy go on to develop dementia within a four year period (De Leon et al., 1997). These data are consistent with human post-mortem histological studies which have shown that the predominant neuronal loss in AD occurs within the CA1 region of the hippocampus, subiculum, and entorhinal cortex (reviewed in Sirinathsinghji, 1998). Since there is clear evidence for neuronal loss and damage within the hippocampus of AD patients, studies into the effects of transgene expression on hippocampal synaptic plasticity may help determine whether the effects of abnormal metabolism of APP alter pre- and/or post-synaptic processes associated with LTP/LTD, and will enable the key neurotransmitter systems that are involved to be identified.

It is likely that changes in synaptic plasticity within the hippocampus are involved in the acquisition and retention of spatial and temporal information. The phenomenon of long-term potentiation is considered by many, but not all, to contribute to this process, and the arguments in favour or against have been reviewed extensively (e.g. Bliss and Collingridge, 1993; Barnes, 1995). It is important to recognise that there are many forms of long-term changes in synaptic plasticity, which differ in the direction of their effects on synaptic strength (e.g. LTP/LTD), their duration, as well as their criteria

Table 4
Effects on hippocampal synaptic plasticity

Transgenic	Assay	Age	Induction paradigm	Region	Paired pulse potentiation	Long-term potentiation		Behavioural deficits
						Early-phase	Late-phase[a]	Spatial/Cued
APP-null	In vitro	12 months	10 × 100 Hz	CA1	⇔[b]	⇔	n.s.[c]	Yes
	In vitro	12 months	4 × 10 at 100 Hz	CA1	⇓	⇓		
	In vitro	24 months	4 × 10 at 100 Hz	CA1	⇓	⇓		
	In vitro	24 months	100 at 100 Hz	CA1	⇔	⇔		
APP(C104) truncated	In vitro		Theta burst	CA1	⇔	⇓	n.s.	Yes
APP695 mutant (K670N + M671L)	In vitro		Theta burst	CA1	⇔	⇓	n.s.	Yes
			Theta burst	DG		⇓		
Down syndrome YAC 152F7	In vitro		2 × 100 Hz	CA1	⇔	⇔	n.s.	Yes
	In vitro		5 Hz (30 s)	CA1	⇔	⇔		
PS-1(A246E)	In vitro		Theta burst		⇔	⇑	n.s.	n.s.
			100 at 100 Hz		⇔	⇑		

[a] Late-phase LTP defined as that lasting >2 h.

[b] ⇔, no change; ⇓, decrease; ⇑, increase.

[c] n.s., not studied

for induction and maintenance. These factors vary according to the brain region studied, the stimulus used, and the neuronal subtypes involved.

6.2. Induction of hippocampal synaptic plasticity

Within the hippocampus at least four forms of synaptic enhancement are recognised and which are based, in part, on their duration. These include paired-pulse facilitation, post-tetanic potentiation, short-term potentiation, and long-term potentiation. In the CA1 region of the hippocampus LTP is critically dependent upon a postsynaptic depolarisation coincident with a rise in intracellular calcium concentration. This induction phase is mediated, in part, by the depolarisation-induced relief of Mg^{2+} block of NMDA receptors and the subsequent influx of extracellular calcium via glutamate gated ion channels (Bliss and Collingridge, 1993).

The type of conditioning stimulus used has a profound influence upon the direction, magnitude, and duration of the change in synaptic strength, and can influence the pharmacological basis of the response. Typical conditioning stimulus frequency paradigms include repetitive tetanic stimulation that usually consist of 1 s bursts of 100 Hz stimuli, theta burst stimulation protocols that consist of short bursts of 100 Hz stimuli every 200 ms, typically four stimuli repeated ten times every 200 ms, and pairing protocols in which different synaptic inputs are stimulated coincidentally with one another. It is important to recognise that each of these different stimulus paradigms will preferentially activate different synaptic pathways, in particular LTP formation induced

by tetanic stimulation can override the influence that GABA-mediated synaptic transmission has upon synaptic integration (Davies and Collingridge 1996; Seabrook et al., 1997). Consequently, the choice of stimulus paradigm may be critical to whether or not one sees changes in synaptic plasticity in transgenic animals and this may have a bearing upon its relevance to cognitive performance.

Surprisingly few studies have been carried out into the effects of transgene expression on hippocampal synaptic plasticity in animal models of AD, however, where this has been studied LTP formation has been found to be disrupted in several cases (Table 4). These include APP-null mice in which behavioural and LTP deficits have been identified (Dawson et al., 1998; Seabrook et al., 1999). In APP-null mice the deficits in synaptic plasticity in the CA1 region were subtle and were evident only following a burst stimulus paradigm, and not following tetanic stimulation. Interestingly, these deficits were not accompanied by changes in baseline synaptic transmission or paired pulse facilitation, although CA1 neurones from APP null mice had an abormal neuronal phenotype and impaired GABA-mediated synaptic transmission. Other transgenic mice relevant to AD that have been studied include those expressing the carboxy terminus of APP (Nalbantoglu et al., 1997), and mice expressing the human APP695(K670N + M671L) early onset AD mutations (Chapman et al., 1997). The deficits in the human APP695(K670N + M671L) mutant transgenic mice are accompanied by age-dependent elevation in Aβ levels and memory deficits (Hsiao et al., 1995, 1996). However, there are examples of transgenic animals

which have spatial learning deficits but do not have any apparent deficits in hippocampal LTP formation. For example, Smith and co-workers have identified two loci from human chromosome 21 using Down syndrome as a model for complex trait analysis (Smith et al., 1997). Transgenic mice generated using yeast artificial chromosomes exhibit spatial learning deficits but do not have abnormalities in hippocampal LTP, using either tetanic (100 Hz) or low frequency stimulation (5 Hz). Since lesions outside the hippocampus, particularly in the entorhinal or frontal cortex can also affect spatial learning it is possible that in this Down syndrome model the hippocampus was not the source of the cognitive deficits.

6.3. Consolidation of hippocampal synaptic plasticity

Following the induction of long-term potentiation several factors are involved in the consolidation of the temporally encoded information within the hippocampus. These include the activation of kinases including protein kinase A (PKA), protein kinase C (PKCβ and γ isoforms), α calmodulin kinase II (αCamKII), and tyrosine kinase, as well as gene transcription following activation of cAMP response element binding protein 1 (CREB1; reviewed in Bliss and Collingridge, 1993; Abel et al., 1998). However few studies, if any, have addressed whether transgenes relevant to early onset AD affect the maintenance phase of hippocampal synaptic plasticity. Late-phases of LTP, which occur after 2–3 h following induction, are likely to involve gene transcription since this form of LTP can be disrupted by anisomycin. Changes in gene expression are also likely to be involved in late-phase LTP, including a decrease in βPKC and increase in CaMKII expression. It is due to the relatively long time course of late-phase LTP that it is neither easily nor frequently studied in detail, however, there is much evidence to suggest from work on transgenic mutant and knockout animals that this form of synaptic plasticity will be an important area for further investigation in transgenic models of AD (Table 4).

Changes in the density or function of G-protein coupled receptors (e.g. muscarinic receptors, GABA$_B$ receptors, and metabotropic glutamate receptors) can alter the synchronisation of neuronal networks involved in the consolidation of memory processes (Whittington et al., 1995), as well as in the induction of LTP and LTD per se (Davies et al., 1991; Bashir et al., 1993; Bolshakov and Siegelbaum, 1994). Furthermore, since it is clear that a number of different kinases, which differ in their sensitivity to cAMP and $[Ca^{2+}]_i$ are involved in the consolidation of changes in synaptic plasticity to late-phase LTP, it will be important to determine whether one or more of these regulatory mechanisms are affected in transgenic animals relevant to AD.

Recently, a number of gene targeted or transgenic mutant mouse strains have been generated that exhibit impairments in synaptic plasticity and behaviour (reviewed by Chen and Tonegawa, 1997). Some of these animal models are likely to contribute our understanding of the mechanism of that contribute to the deficits found in AD patients. Evidence suggests that deficits in NMDA receptor-dependent LTP in the CA1 region of the hippocampus after high frequency stimulation are correlated with impairments in spatial learning and memory (reviewed by Stevens, 1996). Knockout mice have been also generated in which the NMDA-receptor type 1 gene has been deleted in a regionally restricted manner to subfields of the hippocampus, using the cre-loxP and homologous recombination systems (Tsien et al., 1996a). These mutant mice which preferentially lack NR1 receptors in the CA1 region of the hippocampus at young ages, exhibit NMDA-receptor dependent and CA1 specific deficits in LTP, which are correlated with deficits in spatial memory tasks (McHugh et al., 1996; Tsien et al., 1996b). Conventional, knockin and inducible transgenic technology has been also successfully applied to establish the pivotal role of αCaMKII in synaptic plasticity and spatial learning and memory processes. Transgenic mice either lacking αCaMKII, or carrying an activated form of the αCaMKII by mutating residue Thr286 to an Asp286, or an inactive form by mutating Thr286 to Ala286 exhibit distinct deficits in LTP, impairments in spatial learning and memory, and the representation of space by the hippocampus (Silva et al., 1992a,b; Mayford et al., 1995, 1996; Rotenberg et al., 1996; Cho et al., 1998; Giese et al., 1998).

7. The future

One of the key questions for therapeutic intervention in AD is whether the progression of AD pathology can be reversed, or at least halted. One model that might help answer this question is a conditional transgenic mouse, where the APP transgene is switched off after the mouse has developed the AD pathology. The tetracycline inducible system (Gossen and Bujard, 1992; Furth et al., 1994) is currently the most promising system to achieve temporal control of transgene expression. However, another important question is whether age is a prerequisite to AD as recent evidence may suggest: microinjection of fibrillar Aβ in the cerebral cortex of old, but not young rhesus monkeys has been shown to result in phosphorylation of tau, proliferation of microglia, and neuronal loss proximal to the injection site (Geula et al., 1998). To mimic this situation in a murine model a conditional transgene would be needed that will be switched on after the transgenic mouse has reached a certain age, e.g. 1 year, and then the time taken for the appearance of AD-like pathology, includ-

ing elevated Aβ levels, plaque deposition, behavioural deficits, and neuronal loss could be assessed to determine whether its onset is faster in older animals than in conventional transgenic animals.

The embryonic lethal phenotype of the PS-1 knockout mice demonstrates an important function of presenilin-1 in development. A conditional knockout, in which PS-1 is allowed to function normally during development, but is deleted in the adult, would give further insight into the role of PS-1 in the adult animal, and thereby its role in the regulation of APP processing. It might also answer the question of whether presenilin-1 is a potential target for therapeutic intervention of amyloidogenesis, or if this would compromise an essential function, even in the adult animal, in the regulation of membrane trafficking in the endoplasmic reticulum. Another future direction in the field is to combine the existing APP and PS-1 mutant transgenic lines with other AD risk factors, e.g. SOD, ApoE4, tau, etc. (Carlson et al., 1997). This could further accelerate the onset of AD-like pathology, or promote the development of other features of AD such as neurofibrillary tangles and neuronal loss which are not seen in most of the existing transgenic models.

The development of new transgenic strains relevant to AD has rapidly outpaced their functional characterisation, particularly that concerned with the functional consequences of transgene expression on synaptic plasticity. This is partly because of the resources needed to evaluate each strain in sufficient detail but also because many studies have focused on the characteristics of mice at relatively young ages. Future studies will need to answer the questions of which neuronal subtypes are most affected and how synaptic plasticity is disrupted in models of AD. Furthermore, it remains to be resolved whether the deficits are specific to the induction process or whether they also affect the maintenance phase of LTP. The locus of deficits in synaptic plasticity will have implications as to whether agents targeted at promoting the induction, or augmenting the maintenance phase, of LTP will be effective as cognition enhancers that could be used as palliative therapies in senile dementia.

Despite the limitations of rodent models for Alzheimer's disease it is clear that the current transgenic and knockout lines of APP and PS-1 have provided, and will continue to provide a unique insight into the degenerative processes underlying AD. Our understanding of the factors that underlie early and late-onset AD will be further advanced with improved models that address the questions of the age-dependence of AD-like pathology, as well as by studies that identify the cause of the deficits in hippocampal synaptic plasticity and cognitive function.

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